REMARKS/ARGUMENTS

Applicant has filed this response to the Office Action dated November 9, 2010. Claim 3 is pending for prosecution. Claim 3 is independent and has been amended. Applicant respectfully requests the withdrawal of all outstanding rejections and objections and the allowance of all pending claims.

I. Claim Rejections - 35 U.S.C. § 103

A. Obviousness

When determining the question of obviousness, underlying factual questions are presented which include (1) the scope and content of the prior art; (2) the level of ordinary skill in the art at the time of the invention; (3) objective evidence of nonobviousness; and (4) the differences between the prior art and the claimed subject matter. Graham v. John Deere Co., 383 U.S. 1, 17-18, 148 USPQ 459, 467 (1966). Moreover, with regard to the last prong of the *Graham* inquiry, "[t]o determine whether there was an apparent reason to combine the known elements in the way a patent claims, it will often be necessary to look to interrelated teachings of multiple patents; to the effects of demands known to the design community or present in the marketplace; and to the background knowledge possessed by a person having ordinary skill in the art. To facilitate review, this analysis should be made explicit." KSR International v. Teleflex Inc., 127 U.S. 1727 (2007).

The person of ordinary skill in the art is a hypothetical person who is presumed to know the relevant prior art. Custom Accessories, Inc. v. Jeffrey-Allan Indus., Inc., 807 F.2d 955, 962, 1 USPQ2d 1196, 1201 (Fed. Cir. 1986). The level of ordinary skill in the art in this area may be determined by looking to the references of record. In re GPAC, Inc., 57 F.3d 1573, 35 USPQ2d 1116 (Fed. Cir. 1995). The references of record in this case reveal that a moderately high level

of sophistication is present in the subject area of the subject area of the instant application. Thus, Applicant submits that, as substantiated by the cited references, those with a bachelor's degree in food technology or the like would most likely be a person with ordinary skill in this field of endeavor.

With respect to objective evidence of non-obviousness, the Applicant submits that the record supports the conclusion that there are long-felt but unsolved needs met by the present invention. As evidenced by the attached Declaration of Dr. Stephanopoulos (the "Declaration"), there is no evidence in the cited prior art of a method to produce ready-to-eat meat products based on entire-muscular tissue, wherein olive oil has been stably incorporated. There was certainly a need in the industry for this technology. There was not a lack of interest in the development of such products, but rather the technological difficulties implicated in the making of these types of products were present. Instability in the incorporation of oil is indeed expected to result in the phenomena stated by the Applicant in paragraphs [0008]-[0009] of App. 10/577,659. The claimed invention has thus addressed a long-felt need in the industry and succeeded to achieve this goal. For at least this reason the Applicant respectfully submits that the claimed invention is not obvious in view of the cited references.

Finally, prima facie obviousness requires that there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the references. This motivation-suggestion-teaching test informs the *Graham* analysis. "To reach a non-hindsight driven conclusion as to whether a person having ordinary skill in the art at the time of the invention would have viewed the subject matter as a whole to have been obvious in view of multiple references," there must be "some rationale, articulation, or reasoned basis to explain why the conclusion of obviousness is

correct." In re Kahn, (Fed. Cir. 2006). The *KSR International* decision by the Supreme Court has not eliminated the motivation-suggestion-teaching test to determine whether prior art references have been properly combined. Rather, in addition to the motivation-suggestion-teaching test, the Court discussed that combinations of known technology that are "expected" may not be patentable. Stated in the affirmative, therefore, combinations are non-obvious and patentable if unexpected. In the present application, no single prior art reference nor any combination thereof (legitimate or otherwise) meets the claimed limitations of Applicant's invention.

II. Rejection of Claim 3

Claim 3 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Domazakis (U.S. Pub. No. 2003/0049364) in view of Hendricks et al. (U.S. Pat. No. 5,053,237) and Brandt (Marinades "Meat" Challenges publication). For the following reasons, Applicant respectfully requests reconsideration and withdrawal of this rejection.

The Examiner asserts that Domazakis teaches most steps of the method as claimed in claim 3 of the present invention. The Examiner admits that Domazakis does not teach the "entire muscular tissue" in relation to the meat product of the present invention. The Examiner asserts that Hendricks teaches this aspect of the present invention. The Applicant will first show how Domazakis deals with different types of meat products and therefore is inapplicable and then will discuss how Hendricks combined with Domazakis does not disclose each and every limitation of the present application. Generally, the prior art alone nor in combination does not (1) brine the entire muscular tissue, (2) tumble the entire muscular tissue, and then (3) tumble the entire muscular tissue with olive oil in order for the olive oil to be absorbed by the muscular tissue.

On page 4 of the Office Action dated 4/21/2011, the Examiner asserts that "one of

ordinary skill in the art would have been motivated to modify Domazakis and to employ the process of incorporation of olive oil in the 'entire muscular tissue' meat pieces as disclosed by Hendricks [..]. One of ordinary skill in the art would have been motivated to do so, in order to increase consumption of health-beneficial unsaturated fats". The Applicant respectfully disagrees with the Examiner in several aspects. For reasons set forth herein, a person of ordinary skills could not reach the claimed method underlined in the present application by modifying Domazakis and adopting the process as disclosed by Hendricks, without exercising additional experimentation.

Domazakis describes a process for the preparation of ***emulsion-type meat based products*** made of ***thin-chopped meat***, that includes the step of adding olive oil after the thin-chopped meat has been mixed with water, *salt, polyphosphoric salts, preservatives, vegetable proteins, milk proteins and starc*h using a ***machine of mixture*** (e.g. a meat grinder or cutter) (see Domazakis paragraphs [0001]-[0007], [0011]-[0012], [0031], [0038]).

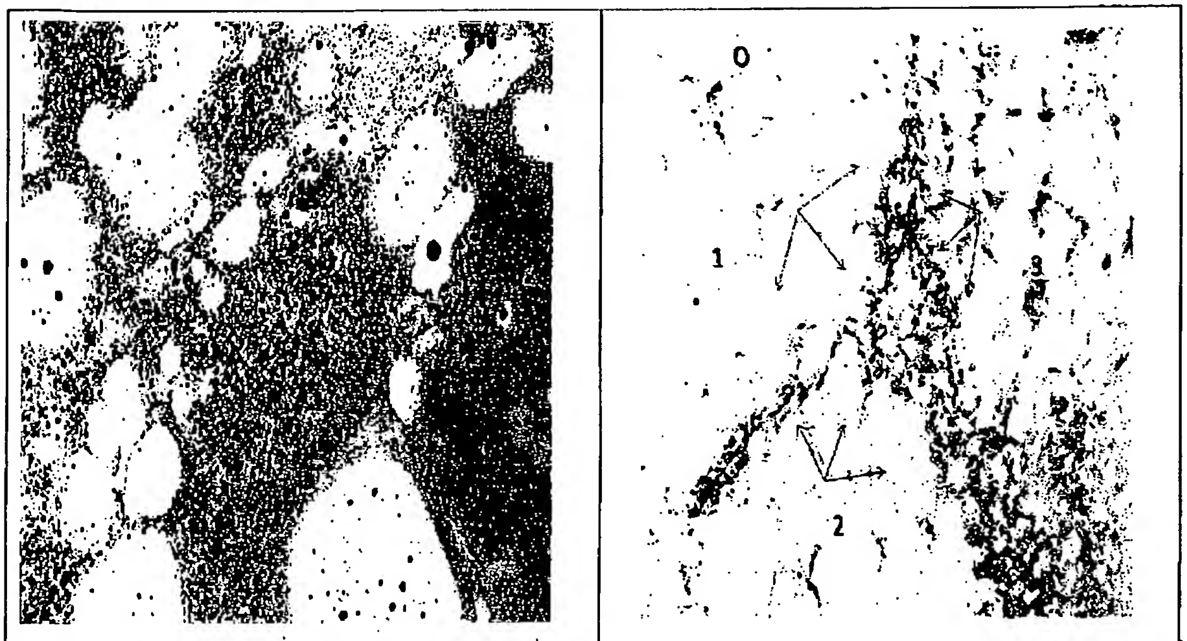

Image 1: **On the left:** Microscopic view (x200) of a section of a meat emulsion-based product, containing olive oil (hot dog), stained with Nile Red (lipid staining). The lipid droplets are stained in red. **On the right:** Microscopic view (x20). Localization of dispersed oil droplets (stained in red), in a section of entire muscular tissue product, made according to the invention. Numbers indicate three separate pieces of meat and the different arrows, the indicative contact areas between them.

Comminution in combination with salt addition significantly alters the structure of a meat system. As seen from the above image (image 1-left), the oil (and fat) globules are dispersed throughout the mass of a complex meat system, containing soluble proteins, but also insoluble components, such as fragments of muscle fibers and connective tissue. To the contrary, the olive oil droplets, within an olive oil containing- product of entire muscular tissue, made by the present invention, are specifically localized at the surface of the meat pieces and thus, in case of joining together individual pieces of meat (in a mould), the oil droplets are mainly seen at the contact areas of the different meat pieces (image 1-right) (see below for further discussion with regards to this matter).

Domazakis discloses a process for the preparation of emulsion-type meat-based products by:

(a) mixing (i.e. not simply contacting) in a mixing machine, thin-chopped lean meat at temperature of 0°C with water at a temperature of -2°C, salt, poly-phosphoric salts preservatives, vegetable proteins, milk proteins and starch (i.e. no use of a brine),

(b) inserting olive oil and continuing mixing in the mixing machine with simultaneous vacuum until the resulting mixture reaches 4°C,

(c) encasing the meat mixture with simultaneous application of vacuum and pasteurising the encased meat mixture, and

(d) freezing the product in freezing chambers up to 2°C.

The method of the present application differs from Domazakis in at least the following aspects. First, Domazakis uses finely-chopped meat for the production of emulsion-type products (e.g. sausages, such as hot dogs). Second, Domazakis uses a technology for emulsion-type meat products. There is no teaching with regards to the technology of cured "entire muscular tissue"- based products as claimed in claim 3 of the present application. Finally, Domazakis faces the challenge of emulsion stability in emulsion-type products containing oil (image 1). To stably incorporate the oil therein, Domazakis adopts the following problem-to-solution approach:

a. The finely-chopped meat is mixed with a number of additional non-meat ingredients, such as salt, poly-phosphoric salts, milk proteins, vegetable proteins and starch.

b. Olive oil is inserted after the admixture of the aforementioned ingredients (i.e. salt, poly-phosphoric salts, milk proteins, vegetable proteins and starch) and the mixing stops when the temperature is 4°C.

However, Domazakis fails to disclose at least the following features of claim 3 of the present application (also referred to as the '659 App.):

1. *producing* meat products from *entire muscular tissue,*
2. *injecting* entire muscular tissue *with brine,*
3. *tumbling* of the brine-injected entire muscular tissue,
4. *adding olive oil* to the *brine-injected and fully tumbled entire muscular tissue*, and
5. *tumbling after the addition of olive oil (a second independent step).*

On the other hand, Hendricks relates to methods, compositions and apparatus for treating and upgrading the tenderness and sensory qualities of ***fresh red meats***. Hendricks "provides a mechanism whereby low grades of meat maybe be made tender, flavourful and juicy [..]. This is accomplished by injecting the meat with ***appropriate injectates***. Such injectates may include unsaturated vegetable fats such as corn oil, water, and even beef tallow or other saturated fats. It is presently preferred to include a binder in the injectate..[..]". (see Abstract). Hendricks differs from the present application in at least the aspects outlined below.

First, Hendricks achieves an upgraded tenderness and sensory qualities of ***fresh red meats,*** thus improving their market value. The '659 App. relates to entire-muscular tissue based ***processed and Ready-to-Eat products,*** such as ham and turkey fillet (see the '659 App., paragraph [0007]), wherein oil is being stably incorporated. The Applicant, as well as Dr. Stephanopoulos (as evidenced by his Declaration), is of the opinion that a fresh meat piece, e.g. a steak, rib eye and round, as disclosed in Hendricks (e.g. see tables 1- 4) should not be regarded as being comparable (or similar) to a processed, ready-to-eat meat product. It is clear that injecting fresh meat with oil and the method in which an entire muscular tissue-based product

stably incorporates olive oil are two different processes and are therefore not able to be compared. (see the Declaration Sections 6 and 7).

Second, according to the basic technology of cured meat products, based on entire-muscular tissue, the meat pieces (previously injected with brine, comprising salt and curing agent) are subjected to mechanical working (tumbling). Mechanical working loosens the structure of the musculature, breaks up cells, and makes brine absorption easier. This in effect increases the mobilization of the extracted, soluble meat proteins. The solubilised meat proteins migrate to the surface, where they form an adhesive substance (paragraph [0008]).

"Processing" in the entire muscular tissue-based products of the present application, further includes <u>*cooking*</u>. Heat-induced gelation is a complex physicochemical process involving structural and functional changes of the proteins. It includes three stages, i.e. dissociation, thermal denaturation and aggregation. Partial unfolding of the protein structure is accelerated by an increase in temperature, which results in the aggregation of the unfolded regions between protein molecules to form a three-dimensional network. The structural and conformational changes that occur as a result of thermal denaturation enable the soluble meat proteins at the surface of the meat pieces (with myosin being the main representative of salt-soluble meat proteins) to gel. Heat-induced gelation of the adhesive substance at the surface of the meat pieces, helps sticking together pieces of tissue items, in order for the resulting product to attain its desirable morphology. Following a heat treatment step (i.e. pasteurization), the resulting product is usually stored in refrigeration conditions until the end of its shelf life.

As is shown by the above, the process associated with the technology of cured entire-muscle based meat products as claimed in claim 3 of the present application, has nothing to do with the preparation of fresh meat cuts found in the counter of a butcher or of a supermarket.

Therefore, there is a substantial difference between the specific ***Ready-to-Eat product*** (product of the '659 App.) and the ***Ready-to-Cook*** product (product of Hendricks).

Third, Hendricks makes use of an "***Injection apparatus***" (Column 6, line 38), which aims to **inject** certain compositions (i.e. injectates) into pieces of fresh meat (injection as a process is characterised by a number of parameters, including an injection depth). In this regard, the '659 App. does not provide any teaching whatsoever with regards to an injection mechanism delivering a fatty substance at an injection depth, into the mass of a muscular tissue. *The brine injection, as also discussed in our previous communication, has been employed for many years in the meat industry.*

Fourth, the problem faced by Hendricks, as well as the approach-to-solution employed is irrelevant to the present case. As mentioned above, Hendricks aims at upgrading the tenderness and sensory qualities of ***fresh red meats***, with the objective to improve their market value. Hendricks further points out the difficulty in retaining the injectate into the meat piece which, in the absence of a binder, has a tendency to cook out (Hendricks uses added ingredients, such methyl cellulose, a common water retention agent, usually applied as a thickener). Quite the contrary, the present application relates to a method of processed entire muscle-based meat products, wherein oil is stably incorporated, without the need of additives. (See also the Declaration at Section 7).

In the present case, a number of critical features, as underlined in the present invention, allowed for the stable incorporation of oil droplets, within the proteinaceous substance at the surface of the meat pieces. Using the method of the present application, the amount of extracted, salt-soluble meat proteins, (which migrated at the surface of the muscular tissue) successfully functioned as emulsifying agents, that stabilised the dispersed oil globules. Therefore, the oil

droplets are stably incorporated in the resulting cooked product, within the proteinaceous gel at the surface of the pieces of muscular tissue. Therefore, if individual meat pieces, processed according to the invention, are joined together in a mould, this proteinaceous oil-containing gel is seen at the contact area regions of the different meat pieces (image 2).

In summary, the localisation of oil incorporation in the obtained product, characterises the novelty of the '659 App. resulting product.

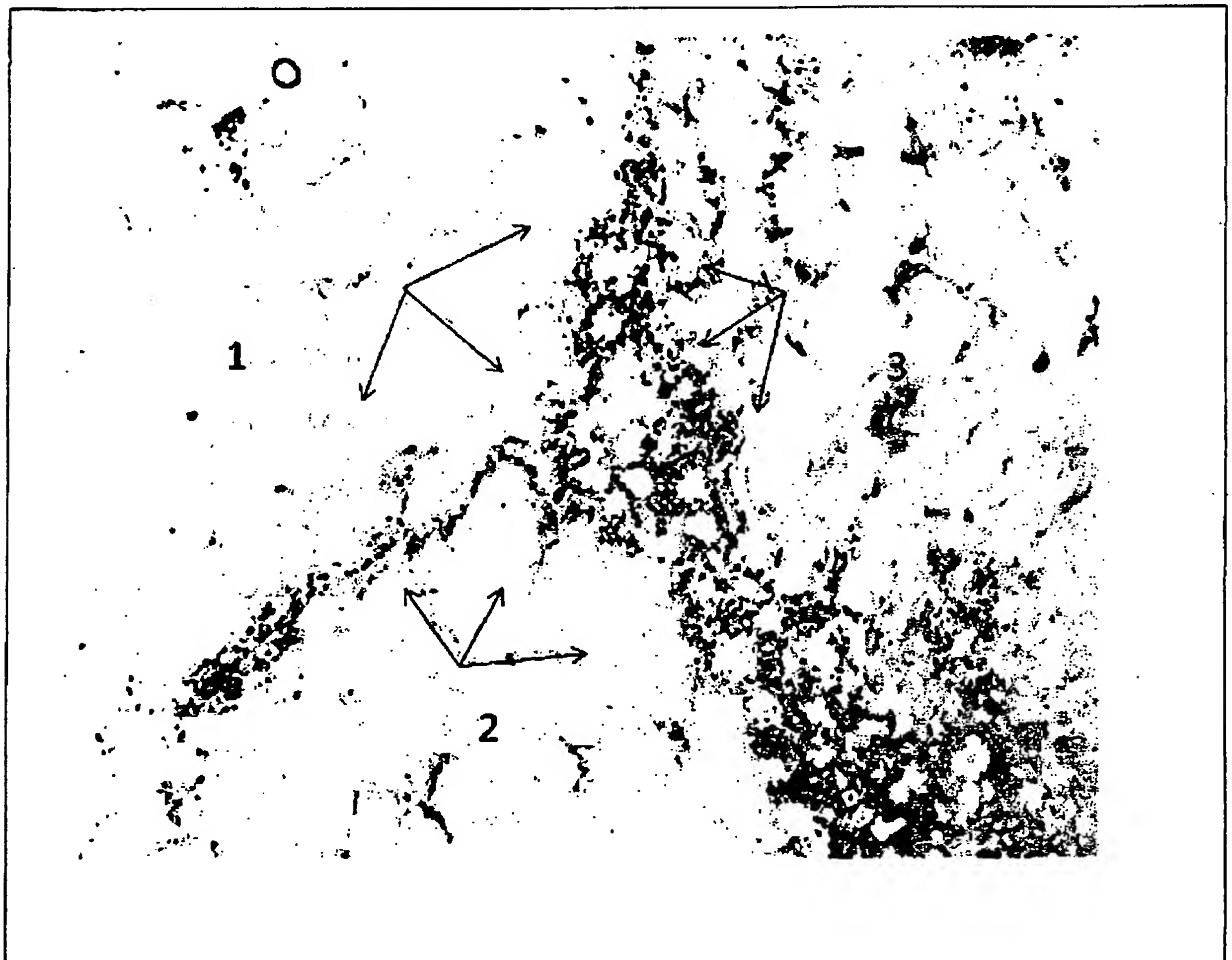

Image 2: (Microscopic view, x20). Localization of dispersed oil droplets (stained in red), in a section of entire muscular tissue cooked product, made according to the invention. Numbers

indicate three separate pieces of meat and the different arrows, the contact areas between them.

This characteristic of '659 App., is neither taught, nor indicated in any of the cited references, either examined individually, nor in combination of such references. With particular reference to Hendricks, the resulting product therefrom is characterised by the deposition/delivery of an injectate (comprising water, oil and a binder) composition that penetrates the mass of fresh meat piece and has nothing to do with the product and method of making such product as claimed in claim 3 of the present invention. (Compare with claim 16 – claiming "injection the meat by causing the injectate to flow from the nozzle at a sufficient pressure such that the injectate composition penetrates the meat and at least a portion of the injectate composition penetrates the existing connective tissue within the meat, wherein the injectate composition being injected into the meat so as to cut at least a portion of the connective tissue within the meat in order to mechanically tenderize the meat").

One of the largest challenges faced by the Applicant in his attempt to develop the said olive oil-containing meat products was the phenomenon of oil exudation and the hindered protein extraction (paragraph [0009]). This was a long and unresolved problem felt in the industry. (See the Declaration). The approach-to-solution, as claimed in the present application, comprises the steps of:

i. *injecting* entire muscular tissue *with brine,*

ii. *tumbling* of the brine-injected entire muscular tissue,

iii. *adding olive oil* to the *brine-injected and fully tumbled entire muscular tissue*, and

iv. *tumbling after the addition of olive oil (a second independent step).*

v. *maintaining a low temperature not exceeding 4°C prior to heat treatment.*

Hendricks is silent with regards to the technical features, underlined in the claimed method of the present patent. Therefore, Hendricks alone or in combination with the other cited references, does not teach each and every limitation of the method claimed in the present application.

The Examiner further asserts that Brandt discloses addition of various ingredients to the meat by either mixing, injecting, tumbling or massaging and that one of ordinary skill in the art would have been motivated to modify Domazakis in view of Brandt and to employ tumbling or injecting instead of mixing as an alternative technique for addition of various components to the meat. The Applicant kindly notes that "mixing, tumbling and massaging of meat" are not presented in Brandt as alternative methods to add various ingredients to the meat, but are rather used to "facilitate tenderization through disintegration of the muscle fiber sheath and stretching of the myofibrils." (see Brandt, page 2 of 7). Even if that was the case, Brandt clearly refers to a "marination system" or "marinating solution" that can be as simple as salt, phosphates and water, or more complex with flavours, seasonings, starches, vegetable or dairy proteins, acids, antimicrobials and antioxidants. The marinating solution/system neither in its simple, nor in its complex formulation, refers to a fatty substance, let alone olive oil in the exemplary amounts disclosed in the present application. Brandt points out that one of the keys to creating a functional marination system is to include ingredients that promote the capability of the muscle to bind water, such as salt and phosphates and those that actually bind water such as soluble proteins and starches. Therefore, by reading Brandt, the person of ordinary skills, is directed away from the use of a fatty substance, such as olive oil in the marinating solution. Further evidence is presented in the Declaration at Section 8. Moreover, Brandt refers to marinated fresh meat pieces, rather to cooked, ready-to-eat products.

The role of a marination system, as correctly addressed by Brandt, is to promote the capability of the muscle to bind water. In order to retain as much as possible of the added water (i.e. to increase product yield, tenderness and juiciness), proper compounds must be blended in the solution of the injectable brine. Sodium chloride, sodium phosphates (typically pyrophosphate), polysaccharide gums, soy or whey proteins, modified starches are common ingredients. The presence of these highly charged or hydrophilic, individual or mixed, compounds enables the injected meat to effectively retain water, thus increasing its yield and palatability. Notably, phosphates can very effectively increase charge repulsions between myofilaments and facilitate the removal of transverse myofibrillar proteins, which act as structural constraints to myosin extraction. The expansion of the myofilamental lattices allows water-binding and physical entrapment in the brine-treated meat. On the other hand, olive oil is a non-polar substance. It is not thus expected to raise the beneficial effect of increasing the water-binding capacity. Most importantly, it is common fact, that olive oil, being non-polar, will not dissolve in the water, as the water molecules will hydrogen bond to each other and will not allow the oil molecules between them.

According to the Applicant' s experimental work, the addition of oil, if it is carried out by the wrong manner and/or at the wrong timing, it is found to result in a number of undesirable phenomena. Inventive work was thus needed to develop the appropriate method, for the attainment of the desirable technical effect. One of the critical process features with a significant technical effect was the precise timing for olive oil addition, i.e. after the brine-injected meat has been <u>fully tumbled</u> (see Claim 3 (b), as originally filed: <u>at the end</u> of the tumbling, the olive oil is added). At that timing, the available amount of extracted meat proteins has been maximized due to the mechanical working of the meat in the tumbler. The solubilised meat proteins migrate to

the surface of the meat pieces and form an adhesive film that, in the present application, not only provides a sticky substance for binding the pieces of meat, in order to attain its desirable morphology, but also offers the appropriate matrix for the emulsification/entrapment/encapsulation of the oil droplets. The addition of olive oil at a wrong timing was found to result in the formation of an insulating layer that hinders the extraction or/and migration of the solubilised meat proteins to the surface of the meat pieces (see the '659 App. paragraph [0008]: "The admixture of fat hinders considerably the injection of various salts (e.g. nitrites) and the extraction of proteins ..[..].").

In summary, the critical technical features underlined in the claimed method, which are linked to one novel aspect of the '659 App., are absent in the cited prior art cited (i.e. Hendricks, Brandt and Domazakis). Even if a skilled person would have attempted to combine the teachings of Hendricks and Domazakis, he would still not arrive at the subject matter of '659 App. In particular, either taken individually or in combination, Domazakis and Hendricks fail to disclose certain critical technical steps, which led to the contribution to the art and the surprising effect of the claimed method, in particular (1) *adding olive oil* to the *tumbled and brine-injected entire muscular tissue*, and (2) *tumbling after the addition of olive oil (a second independent step).* (See the Declaration Section 8). By combining the cited art, the skilled person would have rather considered to use an injectate, the way disclosed in Hendricks, having as a "binder" (see Hendricks, e.g. column 6, lines 23-25) a composition comprising a combination of ingredients as disclosed in Domazakis (i.e. preservatives, polyphosphates, vegetable proteins, milk proteins and starch). There is no such disclosure in the '659 App. Following Brandt, in combination of Domazakis and Hendricks would still not go the person of ordinary skills any further, as it would only teach him how to optimise the marinating solution and employ different methods to

facilitate tenderization. Most importantly, for the reason that Brandt clearly teaches that "All of the ingredients should be dispersed in ambient temperature water for proper dissolution" (see page 2, third paragraph), the person of ordinary skills would rather not consider the said reference any further.

In summary, the method and product derived therefrom taught by the claimed method of the present invention is also novel and inventive, in view of the cited prior art because (1) Domazakis relates to an emulsion-type product, containing olive oil; (2) Hendricks discloses fresh meat pieces having been injected (i.e. delivery of a liquid into the muscular tissue at an injection depth) with an injectate, that preferably also includes an added ingredient, such as methyl cellulose, a conventional water retention agent, often used as a thickener); and (3) Brandt describes marinating solutions for the preparation of fresh marinated meat pieces. The marination solution contains ingredients that promote water binding capacity.

Applicant therefore respectfully submits that neither Domazakis nor Hendricks nor Brandt nor any legitimate combination thereof teaches or suggest all of the limitations of claim 3.

III. Conclusion

Applicant respectfully submits the claims and the application are in condition for allowance and such is courteously solicited. If any issue regarding the allowability of any of the pending claims in the present application could be readily resolved, or if other action could be taken to further advance this application such as an Examiner's amendment, or if the Examiner should have any questions regarding the present amendment, it is respectfully requested that the Examiner please telephone Applicant's undersigned attorney in this regard. Should any fees be necessitated by this response, the Commissioner is hereby authorized to deduct such fees from Deposit Account No. 11-0160.

Respectfully submitted,

Date: 6-17-2011

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: DOMAZAKIS, Emmanouil	:	Examiner: STULII, Vera
Serial No.: 10/577,659	:	Group Art Unit: 1781
Filed: May 1, 2006	:	Attorney Docket No.: 506845.3
For: Method of production of meat products from entire muscular tissue, with direct incorporation of olive oil	:	Customer No.: 27526 Confirmation No.: 8474

Via EFS-Web

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

DECLARATION OF GEORGE STEPHANOPOULOS PURSUANT TO 37 C.F.R. § 1.132

1. I am currently the A. D. Little Professor of Chemical Engineering at the Massachusetts Institute of Technology. My PhD is in Chemical Engineering and I have been in this position for 27 ½ years and have been involved with teaching, research, technology development, and industrial consulting with more than 50 companies in food processing, chemicals, pharmaceuticals, etc. My expertise is in process engineering and I have been involved with a very broad variety of process-product combinations in the food industry and the other industrial sectors mentioned above. I have also worked as Chief Technology Officer for the Group of companies of Mitsubishi Chemical Corporation in Tokyo, Japan, where for 5 years I was in charge of R&D and technology for new business. In this capacity I was the Managing Officer responsible for the Intellectual Property Department of the Corporation and was responsible for Patent Strategy and Patent Defense.

2. I am an author or co-author of many publications. These include:

A. Authored-Coauthored Books

1. "Synthesis of Heat Exchanger Networks," in *Industrial Energy Conservation*, E. Gyftopoulos (Series Editor), MIT Press (1982).

2. *Chemical Process Control: An Introduction to Theory and Practice*, Prentice-Hall (1984). Also in Greek and Chinese translations

3. *Solutions Manual; Chemical Process Control: An Introduction to Theory and Practice*, Prentice-Hall (1985).

4. *Analysis & Planning of Greek Petrochemical Industry*, KEPE, Athens (1986).

5. *The Scope of Artificial Intelligence in Process Engineering,* CACHE Monoghraph (1990).

6. *Intelligent Systems in Process Engineering: Paradigms for Product and Process Design,* by George Stephanopoulos and Chonghun Han, Volume 21 in the "*Advances in Chemical Engineering Series*", Academic Press (1995).

7. *Intelligent Systems in Process Engineering: Paradigms for Process Operations and Control,* by George Stephanopoulos and Chonghun Han, Volume 22 in the "*Advances in Chemical Engineering Series*", Academic Press (1995).

B. Edited-Coedited Books

1. "*Artificial Intelligence in Chemical Engineering Research and Development*" (Geo. Stephanopoulos and M. Mavrovouniotis, Editors), Special Issue of *Computers and Chemical Engineering*, Pergamon Press (1988).

2. *CACHE Case-Studies Series in "Knowledge-Based Systems in Process Engineering"*, 3 Volumes. CACHE (1988).

3. *CACHE Monograph Series in "Artificial Intelligence in Process Engineering"*, edited with J. Davis, 3 Volumes published, 2 in preparation. CACHE (1990).

4. *Foundations of Computer Aided Process Design*, J. J. Siirola, I. E. Grossmann and Geo. Stephanopoulos (editors), CACHE-Elsevier (1990).

5. *On-Line Fault Detection and Supervision in the Chemical Process Indistries*, P.S. Dhurjati and Geo. Stephanopoulos, *IFAC Symposia Series*, No.1 (1993)

6. *ISPE '95: Intelligent Systems in Process Engineering*, Geo. Stephanopoulos, J.F. Davis, and V. Venkatasubramanian (editors), *AIChE Symposium Series*, Vol. 92 (1996)

7. *Proceedings of the European Symposium on Computer-Aided Process Engineering, ESCAPE-6*, Volumes 1 and 2, Geo. Stephanopoulos (editor), *Computers and Chemical Engineering*, (May 1996)

8. *Selected Papers- ESCAPE-6*, Special Issue of *Computers and Chemical Engineering*, Geo. Stephanopoulos and E. Kondili (editors) (1998)

9. *IFAC Proceedings: Dynamics and Control of Process Systems-2001*; Geo. Stephanopoulos, J.H. Lee, and En Sup Yoon, editors. Pergamon Press, 2001.

C. Papers Published in Refereed Scientific Journals: 214

D. Papers Published in Conference Proceedings: 185

3. This Declaration is being presented by me in furtherance of the prosecution of the above-referenced application.

4. I have reviewed the above-referenced application in detail as well as Domazakis (U.S. Pub. No. 2003/0049364), Brandt (Marinades "Meat" Challenge publication) and Hendricks et al. (U.S. Pat. No. 5,053,237), which have been cited during prosecution. I have compared the

method presented in the cited references to the method of the invention disclosed and now claimed in the present application, herein referred to as "App. 10/577,659." After reviewing these references, it is my firm conviction that these references do not render the claimed invention obvious.

5. Although vegetable oil-containing meat products of emulsion-type, may be retrieved in the literature (Dubanchet, U.S. Pat. No. 5,238,701; Bloukas & Paneras[1], 1993, attached hereto as Exhibit A), no evidence has been provided so far with regards to processed, ready-to-eat meat products based on entire-muscular tissue, wherein olive oil has been stably incorporated. This, by no means, indicates a lack of interest in the development of such products, but rather confirms the technological difficulties implicated in the making of these types of products. Instability in the incorporation of oil is indeed expected to result in the phenomena addressed by the Applicant in page 1, lines 32- 44 of App. 10/577,659. The claimed invention has thus addressed a long-felt need in the industry and succeeded to achieve this goal.

6. There is nothing in the cited references themselves or in the knowledge generally available to a person of ordinary skill in the art, at the time App. 10/577,659 was filed, that would lead one of ordinary skill in the art to combine the cited prior art. First of all, the only prior art that at least indicates combination of entire muscular tissue and vegetable oils is Hendricks, yet the goal of the invention, the method followed and the products resulting therefrom, have nothing to do with the goal, the claimed method and resulting products of the present application. Clearly, the goal in Hendricks is to upgrade the tenderness and sensory qualities of fresh red meats, thus improving their market value. However, the deposition of oil inside the mass of a fresh raw meat, by means of an injection apparatus, is substantially different

[1] J. G. Bloukas & E.D. Paneras. *Substituting olive oil for pork backfat affects quality of low-fat frankfurters*, Journal of Food Science, vol. 58 (4), 1993

to the stable oil incorporation, as achieved by the method described in the present patent, in a sliceable ready-to-eat meat product based on entire-muscular tissue. In the latter case, the mechanical working (=tumbling), as well as the presence of sodium chloride, have led to the extraction and solubilisation of myofibrillar proteins, which, surprisingly, were found capable of forming a stable composition on the surface of the meat pieces with the added oil and the free water (by means of emulsification and/or entrapment phenomena). That was an interesting and surprising effect. It is, therefore, the precise localization of the stably dispersed oil droplets, that characterizes the uniqueness of the product resulting from the present application. The novel aspect of App. 10/577,659 is reflected in the description of the critical process features, which allowed for the stable incorporation of the oil droplets in the precise location. In my opinion, neither the precise localization of the dispersed oil globules, nor the critical process features which contributed to the novel aspects of this invention, may be derived from the cited prior art, even if this is considered by the combination of the different references.

7. Hendricks relates to injected pieces of fresh raw meat, which is intended for home cooking. Hendricks merely discloses the use of an "injectate", which is disclosed as a composition that penetrates, by means of pressure injection, the muscular tissue, obviously at an injection depth. Retainment of the delivered injectate, comprising oil, within the muscular tissue was rather challenged, due to the non-stable incorporation of the injectate within the meat mass. The addition of a binder in the composition improved the retention of the injectate. It is thus evident that the physicochemical mechanisms that underline the oil incorporation in the *cooked processed product* of App. 10/577,659, are nowhere disclosed, nor even indicated in Hendricks. The function of "activated" myofibrillar proteins at the surface of meat pieces, which is of primary significance in the mechanism of oil incorporation in App. 10/577,659, is absent in

Hendricks. Rather, Hendricks uses added ingredients, such as non-meat ingredients (e.g. methyl cellulose) to retain the injectate *within* the meat mass. Moreover, the characteristic localization of the dispersed oil phase, as well as the critical process features that ensure the stable incorporation thereof, in the cooked processed product, could not be derived by Hendricks. In my opinion, Hendricks would not even been considered by a person skilled in the art, dealing with the making of *processed ready-to-eat* entire muscular tissue-based cooked products. Moreover, to the extent of my knowledge, I do not recall having seen products resulting from the patented method of Hendricks.

8. In my opinion it would not make sense to one skilled in the art to combine any of the remaining prior art with Domazakis since Domazakis describes the admixture of oil in a finely comminuted meat paste, along with other added ingredients (e.g. phosphates, non-meat proteins and starch) and Brandt describes some basic technological issues regarding marinating fresh meat pieces, such as the use and composition of a marinating solution. Brandt refers to products, such as the Hatfield Marinated Fresh Pork, which are made by injecting a 10% solution, followed by massaging and vacuum packaging (Brandt, page 6 of 7). In fact, Brandt teaches away from the addition of a "non-soluble to water" ingredient, if his instructions should be considered (Page 2 out of 7, 3rd paragraph: "All of the ingredients should be dispersed in ambient temperature water for proper dissolution.") Therefore, Brandt does not teach anything about a fatty substance, let alone olive oil.

9. To my opinion, the cited prior art, either examined individually or in combination, does not provide the critical technical features of the claimed method of App. 10/577,659, including (i) *adding olive oil* to the fully *tumbled and brine-injected entire muscular tissue*, and (ii) proceeding to a second independent *tumbling step after the addition of olive oil.*

10. Accordingly, it is my opinion that the present invention is unique and not obvious based upon my experience in the industry, in view of the unsolved and long-felt need in the industry, and the cited references.

11. I declare that all statements made herein are of my own knowledge are true and all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements and the like are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and such willful, false statements may jeopardize the validity of any patents issued from the patent application.

June 17, 2011
Date

George Stephanopoulos
George Stephanopoulos

EXHIBIT A

Substituting Olive Oil for Pork Backfat Affects Quality of Low-Fat Frankfurters

J.G. BLOUKAS and E.D. PANERAS

ABSTRACT

Low-fat frankfurters (10% fat), formulated for 10%, 12% and 14% protein, were made with olive oil. Compared to control (27.6% all animal fat, 10.9% protein) they had similar flavor, lower ($P<0.05$) TBA values and reduced (44.7–47.6%) caloric content, but had lower ($P<0.05$) processing yield (5.5–6.5%) and overall palatability. Among low-fat treatments, samples with 12% protein had better-quality characteristics. The 12% protein frankfurters compared to the control (except for palatability), had similar ($P>0.05$) sensory attributes and higher ($P<0.05$) skin strength and improved texture. The treatment with 10% protein had undesirable color and was very soft. That with 14% protein had the same ($P>0.05$) red color as the control but higher ($P<0.05$) firmness, skin strength and textural traits and lower ($P<0.05$) juiciness.

Key Words: olive oil, frankfurters, fat substitution, low fat, meat products

INTRODUCTION

IN MOST industrialized societies consumers are recommended to reduce energy intake and to reduce fat intake to 30% or less of total caloric intake (AHA, 1986). Manufacturing calorie-reduced foods, which include low-fat meat products, is of both economic and health interest (Wirth, 1988). Frankfurter type sausages produced with pork fat have up to 30% fat. Pork fat has about 40% saturated fatty acids (Briggs and Schweigert, 1990) while cholesterol is the most important sterol present.

Saturated fat is considered a primary cause of hypercholesterolemia (Mattson and Grundy, 1985) and oxidation products of cholesterol also have adverse human health effects (Pearson et al., 1983; Addis, 1986; Maerker, 1987). Although polyunsaturated fatty acids decrease plasma LDL-cholesterol (Mattson and Grundy, 1985), they promote carcinogenesis in experimental animals (Clinton et al., 1984). In contrast to saturated and polyunsaturated fats, diets high in monounsaturated fat have been associated with decreases in coronary heart disease. Prevalence of heart disease was relatively low in areas of the Mediterranean region in which diets high in monounsaturated fat are typically consumed (Keys, 1970; Keys et al., 1986; Aravanis and Dontas, 1978). Thus incorporation of monounsaturated fats in meat products may have a positive effect on consumer health.

St. John et al. (1986) increased the monounsaturated/saturated fatty acid ratio in low-fat frankfurters using the lean and fat from pigs fed elevated levels of canola oil which contains 64% oleic acid. Shackelford et al. (1991) studied the acceptability of low-fat frankfurters as influenced by feeding of elevated levels of monounsaturated fats to growing-finishing swine. They reported that the high-oleate treatments were comparable to the control in all sensory characteristics. Riendeau (1990) incorporated canola oil into smoked sausages and found that fat and calorie-reduced products were acceptable in quality. Park et al. (1989, 1990) studied the properties of low-fat frankfurters manufactured by direct incorporation of high-oleic sunflower oil (HOSO) as a source of monounsaturated fat. They reported that low-fat frankfurters with maximum allowable added water and HOSO could be manufactured without adverse effects on processing yield, texture or sensory properties.

Virgin olive oil is the most monounsaturated vegetable oil. It contains 56.3–86.5% monounsaturated fatty acids, 8–25% saturated and 3.6–21.5% polyunsaturated fatty acids (IOOC, 1984). It also has tocopherols and phenolic substances which act as antioxidants. Olive oil has a high biological value attributed to its high ratio of vitamin E to polyunsaturated fatty acids (Viola, 1970). It also has a lower ratio of saturated to monounsaturated fatty acids and the presence of antioxidant substances at an optimum concentration (Christakis et al., 1980).

Our objectives were to evaluate quality of low-fat frankfurters (<10% fat) produced by direct incorporation of virgin olive oil as a sole source of monounsaturated fat, and to study effects of protein level in the finished product on quality characteristics.

MATERIALS & METHODS

Ingredients and formulation

Commercial frozen beef meat, fresh pork meat and pork backfat were obtained from the local meat market. Partially thawed beef and the fresh pork were trimmed of separable fat to provide extra lean meats. The lean meat and the pork backfat were separately ground through a 12 mm plate and then through a 3 mm plate. The ground meats and pork backfat were vacuum packaged and frozen at −20°C for 1–2 wk until product formulation. Representative samples were analyzed for moisture, fat and protein (AOAC, 1984) prior to freezing. All raw materials were tempered at 0°C for 24 h prior to use.

Virgin commercial olive oil containing 0.71% free fatty acids (as oleic) was pre-emulsified the day of use. Eight parts of hot water were mixed for 2 min with one part sodium caseinate. The mixture was emulsified with 10 parts oil for 3 min (Hoogenkamp, 1989a, b).

Four treatments were prepared (Table 1). The control was produced using only pork back fat formulated to 28% fat and 11% protein. These values represent about the mean fat and protein content of commercial frankfurters in Greece (Bloukas and Paneras, 1986). The

Table 1—Formulation ingredients

Ingredients (g)	Control[a] A	Low-fat treatments[b] B	C	D
Protein (%)	11	10	12	14
Beef lean (1.32% fat)	700	830	1020	1200
Pork lean (3.87% fat)	1000	1170	1430	1700
Pork backfat (78.84% fat)	1700	—	—	—
Olive oil[c]	—	415	405	395
Ice / water[d]	1830	2815	2175	1735
Sodium chloride	85	87	87	87
Sodium nitrite	1	1.2	1.2	1.2
Sodium ascorbate	3	4	4	4
Phosphates	12	12	12	12
Sodium caseinate	60	60	60	50
Starch	200	200	200	200
Seasoning	24	32	32	32

[a] Prepared with pork backfat and formulated for 28% fat and 11% protein.
[b] Prepared with virgin olive oil and formulated for <10% fat and 10%, 12% and 14% protein.
[c] Percent in batter composition: 7.6%, 7.4% and 7.2%, respectively.
[d] Percent in batter composition: 30.9%, 48.2%, 40.1% and 32.0%, respectively.

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other three treatments were produced with olive oil formulated to give a final product with less than 10% fat and 10%, 12% and 14% protein, respectively. In low-fat treatments the added salt was reduced while the amount of seasonings was increased as suggested by Wirth (1988, 1991) and Hoogenkamp (1989b). All treatments were replicated three times from separate meat and fat sources at three different time periods.

Frankfurter manufacture

The partially thawed lean was mixed with curing ingredients and dry chopped for 20–30 sec in a Laska 30L cutter at low speed. After dry chopping about half the water was added in the form of ice and the chopping continued until a temperature of +3°C was reached. At that point the thawed pork backfat, pre-emulsified olive oil, seasoning and other ingredients, together with the remainder of the ice/water, were added and the batter was chopped at high speed until the final temperature reached 12°C.

Immediately after chopping the batter of each treatment was vacuum stuffed into 24 mm diameter Nojax cellulose casings. Each treatment was handlinked at 15 cm intervals and the frankfurters were heat processed and smoked in a smokehouse to internal temperature 72°C (Hoogenkamp 1989a and b, Wirth 1988, 1991). The frankfurters were showered for 15 min and chilled at +2°C for 24 hr. After chilling the frankfurters were peeled, vacuum packaged (vacuum level 650 mmHg) in film pouches with a reported oxygen permeability rate of ≈116cm³/m²/24 hr/1 atm (23°C, 0% RH) and stored in the dark in a cooler at +4°C until subsequent analysis.

Batter properties

Immediately after processing the following parameters of batters were determined: pH was determined with a WTW digital pH meter with corrections for temperature differences. Viscosity was measured immediately after batter preparation with a Brookfield digital viscometer, model DV-II, set at 2.5 rpm and equipped with a spindle No 5. Frankfurters were weighed before heat processing and smoking and after chilling at +2°C for 24 hr. The processing yield (%) was determined from the weights.

Chemical analysis

Representative samples from each treatment were homogenized and analyzed, prior to vacuum packaging (0 week), for percentage moisture, fat (ether—extractable), protein, ash, starch and sodium chloride according to standard AOAC (1984) procedures. Percent added water was also calculated according to AOAC (1984) formula. Sodium nitrite was determined by the ISO (1975) method. All analyses were performed in duplicate.

Purge loss

Two vacuum packages (≈ 250–300g each) per treatment were used to determine purge loss of frankfurters the 1st, 3rd and 5th week of storage in the dark at 4°C. Before packaging each link of frankfurters was dried with paper tissue and all links per package were weighed. After removing sausages from the package each link was again dried with paper tissue and all links per package were reweighed. Purge loss was determined from the difference in weights between the two measurements expressed as percentage of initial weight.

Color measurements

Color measurements were performed the 0 and 5th week of storage. A True—Color Neotec colorimeter was used to evaluate L, a and b (Hunter color system). The instrument was standardized using a white ceramic tile calibrated to tristimulus values of L = + 96.0, a = −1.03, and b = + 2.4. Two frankfurters per treatment were used. The surface of the glass tray was completely covered with sections of the frankfurters and four measurements were taken per link by rotating the glass tray one-quarter after each measurement. Data are means of eight measurements.

Rancidity determination

The 2-Thiobarbituric acid (TBA) test according to Tarladgis et al. (1960) was used to determine extent of oxidative rancidity after the 0, 1st, 3rd and 5th week. Two frankfurters were randomly sampled from each treatment. The frankfurters were ground in a chopper for 1 min and two 10-g portions were removed for TBA analysis. Duplicate determinations were conducted on each treatment. The amount of residual nitrite in each sample was taken into account and the amounts of sulfanilamide were added in the samples for TBA analysis according to the modifications of Shahidi et al. (1985). Readings were made on a LKB Ultrospec II spectrophotometer at 538 nm. The conversion factor 7.8 was used in calculation of TBA numbers.

Sensory evaluation

Sensory evaluation was conducted the 1st and 5th week of storage by a five-member trained panel. The panelists were chosen on the basis of previous experience in evaluating frankfurters. The following attributes were evaluated on a 5-point or 8-point scale: color (5 = very intensive, 1 = very poor), springiness (5 = extremely springy, 1 = not springy), firmness (8 = extremely firm, 1 = extremely soft), juiciness (8 = extremely juicy, 1 = extremely dry), flavor intensity (8 = extremely strong, 1 = extremely weak to unpleasant), overall palatability (8 = palatable, 1 = unpalatable). Each attribute was discussed and tests were initiated after panelists were familiarized with scales. Samples were prepared by steeping frankfurters in boiling water in individual pans 2 min. Warm, 2.5 cm long pieces from each treatment were randomly distributed for evaluation. Tap water was provided between samples to cleanse the palate.

Texture profile analysis

An Instron Universal Testing Machine, model 1140, was used to conduct texture profile analysis, as described by Bourne (1978), after 1 wk storage. Samples were prepared by steeping frankfurters in boiling water for 2 min and cooling to ambient temperature. Four 20 mm long sections per treatment were axially compressed by a two cycle compression test to 75% of original height. Force-time deformation curves were recorded at a crosshead speed 5 cm/min, chart speed 5 cm/min and full scale 50 kg. Texture variables of force and area measurements were: FP = force to fracture; F1 = maximum force for first compression; A1 = total energy for first compression; F2 = maximum force for second compression; A2 = total energy for second compression; springiness (S) = height sample recovered between end of first compression and start of second; gumminess = F1×A2/A1; chewiness = F1×A2/A1×S; and cohesiveness = A2/A1. Peak areas were determined by using the Ladd Graphic Data Analyzing System.

Skin strength

Skin strength of frankfurters was measured with a penetrometer Sur-Berlin, model PNR 6, equipped with a half-scale aluminum cone of 45 g and 20 g load weight. Samples were prepared by steeping frankfurters in boiling water for 2 min and cooling to ambient. The pointed part of the cone was placed at the surface of the frankfurters and the instrument was turned on for 10 sec to produce a puncture. The depth of puncture was measured in mm and higher depth means less skin strength. The same procedure was applied to five surface areas of each of two links of frankfurters per treatment. Data reported are means of ten measurements.

Statistical analysis

Data collected for batter characteristics, processing yield, chemical composition, sensory and instrumental texture profile values were analyzed by one-way analysis of variance. Data collected for purge losses, pH, TBA values and instrumental color were analyzed by a two factor factorial arrangement in a completely randomized design. The factors were: treatments (A,B,C,D) and storage time. Means were compared by using the $LSD_{0.05}$ test. Data analyses were performed using the MSTAT program.

RESULTS & DISCUSSION

MEAN pH and viscosity for uncooked batter of control and low-fat frankfurters containing olive oil were compared (Table 2). No differences ($P>0.05$) were found between pH of control and low-fat batters. The Brookfield viscosity of uncooked batter in low-fat frankfurters was higher ($P<0.05$) in treatments

Table 2—pH and viscosity for uncooked batter of control and low-fat frankfurters containing olive oil[f]

Parameters	Control[a] 11%	Low-fat treatments[b] 10%	12%	14%
pH	6.60 (0.26)[e]	6.61 (0.23)[e]	6.41 (0.12)[e]	6.33 (0.11)[e]
Brookfield viscosity (cp X 10[3])	414 (17.21)[e]	251 (14.93)[e]	339 (69.65)[d]	456 (38.16)[e]

[a] Prepared with pork backfat and formulated for 28% fat and 11% protein.
[b] Prepared with virgin olive oil and formulated for <10% fat and 10%, 12% and 14% protein.
[c-e] Means within the same row with different superscript letters are different ($P < 0.05$).
[f] Means (standard deviation).

Table 3—Processing yield and proximate composition of control and low-fat frankfurters containing olive oil[g]

Parameters	Control[a] 11%	Low-fat treatments[b] 10%	12%	14%
Processing yield (%)	86.6 (3.8)[d]	80.2 (7.2)[e]	80.5 (5.9)[e]	80.5 (4.7)[e]
Moisture (%)	55.0 (0.8)[d]	70.6 (0.4)[e]	68.7 (0.5)[e]	68.0 (0.6)[f]
Protein (%)	10.9 (0.4)[d]	10.7 (0.1)[d]	12.4 (0.2)[e]	14.3 (0.2)[f]
Fat (%)	27.6 (0.7)[d]	11.6 (0.1)[e]	10.6 (0.4)[e]	10.6 (0.7)[e]
Ash (%)	2.6 (0.1)[d]	2.6 (0.1)[d]	2.7 (0.1)[de]	2.8 (0.1)[e]
Starch (%)	3.8 (0.4)[d]	4.3 (0.6)[d]	4.1 (0.8)[d]	4.1 (0.7)[d]
Sodium chloride (%)	1.8 (0.1)[d]	1.8 (0.1)[d]	1.8 (0.1)[d]	1.8 (0.1)[d]
Sodium nitrite (ppm)	112 (8.8)[d]	117 (7.5)[d]	125 (23.0)[d]	110 (13.0)[d]
Added water (%)[h]	12.6 (2.6)[d]	38.8 (0.5)[e]	24.9 (1.4)[f]	11.8 (0.8)[d]
Caloric content (Kcal/100g)[c]	312	163	168	172
Caloric content reduction (%)		47.6	46.1	44.7

[a] Prepared with pork backfat and formulated for 28% fat and 11% protein.
[b] Prepared with virgin olive oil and formulated for <10% fat and 10%, 12% and 14% protein.
[c] Calculations based on 9.1 Kcal/g for fat and 4.1 Kcal/g for protein and carbohydrates (Wirth, 1988).
[d-f] Means within same row with different superscript letters are different ($P < 0.05$).
[g] Means (standard deviation).
[h] Percent added water = [W − 4P]/[1 − 0.01W + 0.04P], where W = moisture %, P = protein % (AOAC, 1984).

with higher protein. No differences were found in viscosity between controls and low-fat treatments with 14% protein. The added water in both treatments was similar, 12.6% and 11.8% respectively (Table 3). These results agreed with Claus et al. (1989) who found that added water had greater effect than fat or protein on Brookfield viscosity.

Processing yields (Table 3) for control (86.6%) were 5.5–6.5% higher ($P<0.05$) than for low-fat treatments (80.2–80.5%). These results were in accordance with Townsend et al. (1971) who found that frankfurters with vegetable oil had lower processing yield than those prepared with animal fat. Preliminary experiments have shown that the small reduction of added salt in low-fat treatments, (16.1g/kg of batter instead of 17.5 g/kg in the control) had no effect on processing yield. Park et al. (1989) also reported that control frankfurters with 30% animal fat had 5–6% higher yield than low-fat treatments with ≈17% oil and the same added salt.

The proximate composition of control frankfurters was very near the targeted values. Total fat and protein concentrations of low-fat frankfurters were higher than targeted values, due to higher moisture loss during processing. For purposes of discussion, references to protein concentrations will be made according to formulated levels. The higher the protein content the lower the moisture content of the low-fat frankfurters except for the frankfurters with 10% and 12% protein where there was no difference ($P>0.05$). No differences ($P>0.05$) were found in sodium chloride and sodium nitrite content although added quantities in low-fat treatments were slightly different.

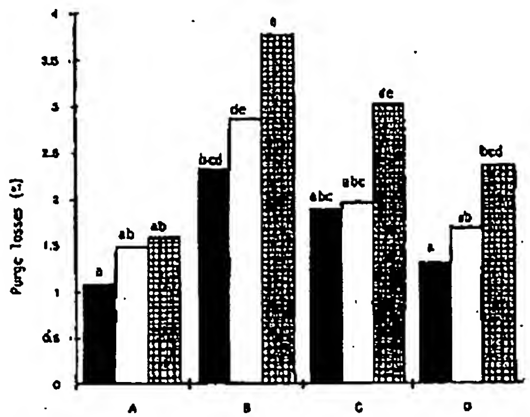

Fig. 1—Effect of storage time on purge losses of control (A) and low-fat frankfurters (B,C,D) containing olive oil. (A) Prepared with pork backfat and formulated for 28% fat and 11% protein. (B,C,D) Prepared with virgin olive oil and formulated for <10% fat and 10%, 12% and 14% protein, respectively. a-e Bars with different superscript letters are different (P<0.05). ■ 1st wk, □ 3rd wk, ▫ 5th wk.

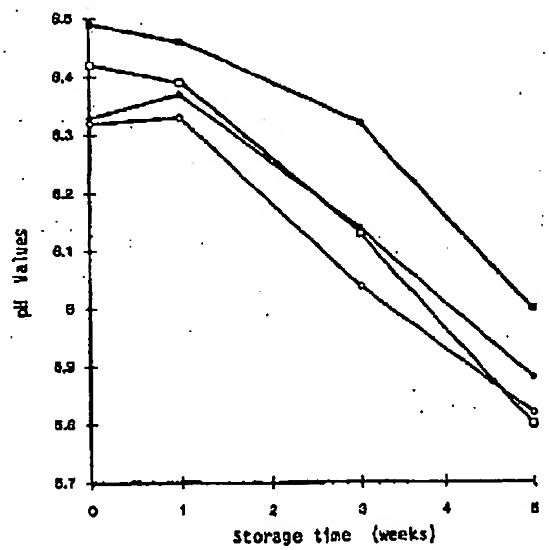

Fig. 2—pH values of control (A) and low fat frankfurters (B,C,D) containing olive oil. (A) ■——■ Prepared with pork backfat and formulated for 28% fat and 11% protein. (B) □——□, (C) ◆——◆, (D) ◇——◇ prepared with only virgin olive oil and formulated for <10% fat and 10%, 12% and 14% protein, respectively.

The total reduction in caloric content of low-fat frankfurters ranged from 44.7% to 47.6% compared to controls.

The low-fat treatment with 10% protein had higher ($P<0.05$) purge loss than all other treatments. Storage time had a significant effect on purge losses, especially in low-fat treatments (Fig. 1). The lower the protein level the higher the purge losses. The low-fat treatment with 14% protein was not different

Table 4—Effect of storage time on TBA values (mg malonaldehyde/kg) of control and low-fat frankfurters containing olive oil

Storage time at 4°C	Control[a] 11%	Low-fat treatments[b] 10%	12%	14%
0 week	0.81^{d}	0.82^{e}	0.59^{e}	0.45^{f}
1st week	0.94^{d}	0.48^{f}	0.55^{g}	0.35^{f}
3rd week	0.97^{d}	0.97^{f}	0.66^{f}	0.82^{e}
5th week	0.85^{d}	0.63^{f}	0.53^{e}	0.42^{e}

[a] Prepared with pork backfat and formulated for 28% fat and 11% protein.
[b] Prepared with virgin olive oil and formulated for <10% fat and 10%, 12% and 14% protein, respectively.
[c-f] Means within same row with different superscript letters are different ($P < 0.05$).

Table 5—Hunter color values of control and low-fat frankfurters containing olive oil

Hunter color numbers	Storage time (wk)	Control[a] 11%	Low-fat treatments[b] 10%	12%	14%
L (lightness)	0	56.0^{d}	55.7^{d}	54.4^{e}	54.2^{e}
	5	54.8^{e}	55.7^{d}	54.2^{e}	53.8^{e}
a (redness)	0	14.4^{d}	11.1^{f}	12.4^{e}	14.7^{d}
	5	13.6^{d}	10.6^{f}	11.8^{e}	14.0^{d}
b (yellowness)	0	12.9^{e}	13.6^{d}	13.2^{d}	13.1^{e}
	5	13.2^{d}	13.8^{d}	13.6^{d}	13.1^{e}

[a] Prepared with pork backfat and formulated for 28% fat and 11% protein.
[b] Prepared with virgin olive oil and formulated for <10% fat and 10%, 12% and 14% protein.
[c-e] Means within rows of same numbers with different superscript letters are different ($P < 0.05$).

Table 6—Sensory scores and instrumental texture profile of control and low-fat frankfurters containing olive oil[c]

Parameters	Control[a] 11%	Low-fat treatments[b] 10%	12%	14%
Sensory attribute:				
Color[g]	4.0^{d}	3.0^{e}	4.0^{f}	4.5^{d}
Springiness[h]	4.2^{d}	4.1^{d}	4.2^{d}	4.3^{d}
Firmness[i]	4.5^{d}	2.7^{e}	4.2^{d}	6.5^{f}
Juiciness[j]	7.2^{d}	6.8^{de}	6.4^{de}	5.9^{e}
Flavor intensity[k]	5.7^{d}	5.6^{d}	5.8^{d}	5.8^{d}
Overall palatability[l]	7.3^{d}	5.7^{e}	6.6^{f}	6.4^{f}
Skin strength (mm)	155.6^{d}	188.0^{d}	120.3^{e}	77.0^{f}
Texture profile:				
Fracturability (FF)[m]	34.0^{d}	48.7^{d}	61.1^{e}	68.0^{e}
1st bite hardness (F1)[m]	47.4^{d}	43.8^{d}	80.7^{e}	109.2^{f}
2nd bite hardness (F2)[m]	32.6^{d}	24.8^{e}	58.5^{e}	87.6^{f}
Springiness (S)[m]	15.1^{c}	12.7^{f}	15.4^{de}	17.0^{e}
Cohesiveness (A2/A1)	0.2^{d}	0.1^{d}	0.2^{d}	0.2^{d}
Gumminess (F1XA2/A1)	9.2^{d}	6.7^{e}	18.4^{f}	23.7^{j}
Chewiness (F1XA2/A1XS)	140.2^{d}	87.6^{d}	264.0^{e}	403.6^{f}

[a] Prepared with pork backfat and formulated for 28% fat and 11% protein.
[b] Prepared with virgin olive oil and formulated for 10% fat and 10%, 12% and 14% protein.
[c] Data presented are means
[d-f] Means within row with different superscripts are different ($P < 0.05$).
[g] 8 = very intensive, 1 = very poor
[h] 8 = extremely springy, 1 = not springy
[i] 8 = extremely firm, 1 = extremely soft
[j] 8 = extremely juicy, 1 = extremely dry
[k] 8 = extremely strong, 1 = extremely weak to unpleasant
[l] 8 = palatable, 1 = unpalatable
[m] Expressed in Newtons

($P>0.05$) in purge loss from the control during the storage period of 5 wk. Claus et al. (1990) found that the low-fat frankfurters had higher consumer shrink and purge losses. Higher purge losses of low-fat frankfurters were due to lower ionic strength. In our experiment the added salt in low-fat treatments was purposely reduced slightly. This probably contributed to further decrease of ionic strength in low-fat treatments. The increase in purge losses during storage was due to the decrease in pH. The correlation coefficient between purge losses and pH after the 1st week of storage was $r = -0.644$ ($P<0.05$). The pH of control was reduced from 6.5 to 6.0 and that of low-fat treatments from 6.4 to 5.8 during the 5 wk storage of vacuum-packed frankfurters at 4°C (Fig. 2). Paneras and Bloukas (1988) reported a decrease in pH from 6.3 to < 5.8 during the 9 wk storage of vacuum packed frankfurters at 3°C. Kempton and Bobier (1970) also found a decrease in pH from 6.3 to 5.4 during storage of frankfurters under vacuum at 5°C for 28 days. Simard et al. (1983) reported a decrease in pH from 6.18 to 5.42 during 7 wk storage of frankfurters under vacuum at 7°C. The pH decrease was attributed to activity of lactobacilli, and/or dissolution of CO_2 into meat tissue.

TBA values of refrigerated vacuum-packaged frankfurters over 5 wk were compared (Table 4). All low-fat treatments containing olive oil had lower ($P<0.05$) TBA values than control, initially and during 5 wk storage. The lower TBA values observed in olive oil containing frankfurters was attributed to tocopherols and phenolic substances with antioxidant activity in addition to nitrite. The TBA values of control treatment although higher than low-fat treatments were lower than acceptable range (<1.0) for oxidative rancidity (Ockerman, 1976). Storage time did not affect TBA values, probably due to the presence of curing ingredients, such as nitrite, phosphate and ascorbate, which also act as antioxidants.

Means for color measurements (Table 5) showed no difference ($P>0.05$) in Hunter L and b values between treatments and storage time. These results were in agreement with Ahmed et al. (1990) who found that decreasing fat content in fresh pork sausages with simultaneous increase in added water, did not affect Hunter L values. The lower the protein level of low-fat frankfurters the lower ($P<0.05$) the redness. The low-fat treatment with 14% protein level had the same ($P<0.05$) Hunter a value as the control. Differences in redness between low-fat treatments were due to different added water and protein levels. In low-fat treatments, added water increased from 12.4% to 39.2% while protein content was inversely reduced from 14.3% to 10.7% (Table 3). Reduced protein content resulted in dilution of myoglobin and consequently less red color. During the 5 wk refrigerated storage under vacuum no decreases in redness were observed.

Data on sensory scores and instrumental texture profiles of control and low-fat frankfurters containing olive oil were compared (Table 6). The low-fat treatment with 10% protein had lower ($P<0.05$) color, firmness and overall palatability scores. The treatment with 12% protein had similar ($P>0.05$) sensory attributes except palatability. The higher the protein content the higher ($P<0.05$) the firmness in low-fat frankfurters. Simon et al. (1965) and Claus et al. (1989) reported the same effects. Differences in flavor intensity between the control and low-fat treatments were not significant.

The 1st week of storage the control treatment had higher ($P<0.05$) overall palatability scores while differences between low-fat frankfurters with 12% and 14% protein were not significant. The frankfurters with 10% protein were very soft while those with 14% protein were harder and less juicy than the control. During the 5 wk cold storage a ($P<0.05$) reduction in overall palatability was found in all treatments (Fig. 3). The control treatment had higher ($P<0.05$) overall palatability while in low-fat treatments containing olive oil the higher the protein level the higher the overall palatability. The observed decrease in palatability during storage was probably due to microbial activity of lactic acid bacteria, which is in agreement with pH reduction (Fig. 2).

The control treatment had higher skin strength and fracturability and not significant changes in bite hardness, gumminess and chewiness with 10% protein low-fat frankfurters. This was probably due to the similar protein level of the 2 treatments

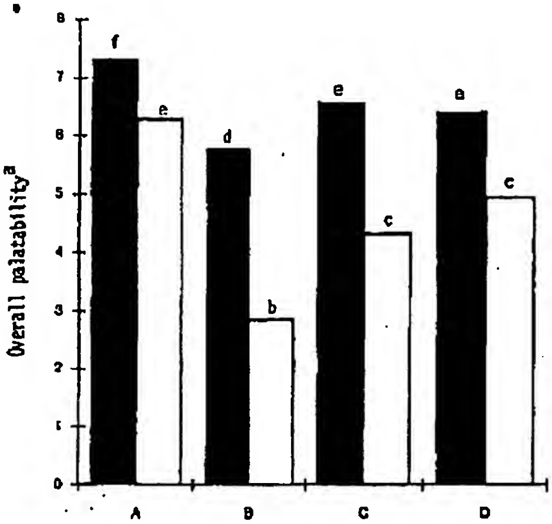

Fig. 3—Overall palatability scores the 1st and 5th week of storage of control (A) and low-fat frankfurters (B,C,D) containing olive oil. (A) Prepared with pork backfat and formulated for 28% fat and 11% protein (B,C,D) Prepared with virgin olive oil and formulated for < 10% fat and 10%, 12%, and 14% protein, respectively. ■ 1st wk. □ 5th wk. [a]8 = palatable, 1 = unpalatable; [a-e] Bars with different superscript letters are different ($P<0.05$).

(Table 3). According to Saffle et al. (1964) the skin strength is developed by the migration of protein to the surface of frankfurters and subsequent denaturation during smoking. Differences between the control and low-fat treatments with 12% and 14% protein for skin strength, fracturability, 1st and 2nd bite hardness, springiness, gumminess and chewiness were significant. The higher the protein in low-fat treatments the higher ($P<0.05$) was the skin strength, the 1st and 2nd bite hardness, gumminess and chewiness. Low-fat treatments with 12% and 14% protein had no significant differences for fracturability and springiness while all treatments had the same ($P<0.05$) cohesiveness.

CONCLUSIONS

LOW-FAT FRANKFURTERS (10% fat) could be manufactured with olive oil and without added animal fat. The low-fat frankfurters would be highly desirable from a diet/health standpoint as they contain monounsaturated vegetable oil, have lower caloric value, reduced cholesterol and a higher protein content. Among low-fat treatments with olive oil, that with ≈ 12% protein had quality characteristics most comparable to the control.

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Ms received 10/23/92; revised 1/26/93; accepted 2/16/93.

Thanks to Dr. S. Raphaelidis of the Technological Educational Institution of Thessaloniki, for textural measurements.